13. Diekema DJ, Andrews JI, Huynh H *et al.* Molecular epidemiology of macrolide resistance in neonatal bloodstream isolates of group B streptococci. *J Clin Microbiol* 2003; 41: 2659–2661.
14. Elliott JA, Farmer KD, Facklam RR. Sudden increase in isolation of group B streptococci, serotype V, is not due to emergence of a new pulsed-field gel electrophoresis type. *J Clin Microbiol* 1998; 36: 2115–2116.
15. von Both U, Ruess M, Mueller U *et al.* A serotype V clone is predominant among erythromycin-resistant *Streptococcus agalactiae* isolates in a southwestern region of Germany. *J Clin Microbiol* 2003; 41: 2166–9.

Genetic analysis of four human rabies cases reported in Turkey between 2002 and 2006

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Abstract

Rabies remains endemic in many regions of Turkey. As a consequence, humans are at risk of this fatal disease through encounters with rabid animals. The present study describes four recent cases of rabies in humans. Subsequent phylogenetic analysis of the rabies virus isolates obtained from each case demonstrates the distinct geographical distribution of rabies virus variants within Turkey. The study suggests that rabies virus translocation has occurred across Turkey and might be the source of the emergence of a genetically similar variant in the Golan Heights region on the Israeli/Syrian border in 2004.

Keywords: Diagnosis, rabies virus, transmission, Turkey

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Rabies is one of the major zoonotic diseases in Turkey. The virus is enzootic in many areas of Turkey, with dogs being the principal reservoir and vector, bringing humans into contact with the virus [1]. The presence of the disease in dogs and, in some areas, in wildlife, represents a continuing threat to public health and causes economic losses, especially in domestic livestock [2]. Within Turkey, veterinary control and research institutes are responsible for diagnosis of the disease (in animals) at sites distributed throughout the country (Adana, Elazıg, Erzurum, Etlik-Central-Ankara, Konya, Istanbul, Izmir, Samsun). The Ministry of Agriculture and Rural Affairs reports to OIE and WHO regularly. Reported cases in animals between 2002 and 2006 were 217, 156, 111, 193 and 210, respectively, suggesting the persistence of the disease (http://www.who-rabies-bulletin.org). The Ministry of Health is responsible for diagnosis of cases of rabies in humans and for subsequent reporting to WHO.

In the present study, we have investigated four recent cases of rabies in humans (Table 1). This is the first report of ante-mortem rabies diagnosis in Turkey; cases TR149 and TR40 tested positive according to the direct fluorescent antibody test (dFAT) on corneal smears and RT-PCR on saliva and eye swabs. It is also the first genetic analysis of rabies viruses of human origin from Turkey.

The first case (TR33) occurred in Kilis province in 2002, the second (TR149) in Agri province in 2004, the third (TR40) in Adana province in 2006, and the fourth (TR3) in Aydin province in 2006. For each case, brain tissue specimens, saliva, corneal impressions, swabs from the eyes, and cerebrospinal fluid were collected. Samples were shipped to the National Reference Laboratory for rabies at the Etlik Central Veterinary Control and Research Institute (ECVCRI) for further investigation. Infection with rabies was confirmed by dFAT. The test was performed using a commercial conjugate (Fujirebio, Malvern, USA) as described previously [3]. The mouse inoculation test was undertaken using standard protocols [4]. Total RNA was extracted directly from samples by the TRIzol method according to the manufacturer's instructions (TRI® Reagent; Sigma-Aldrich ChemieGmbH, Germany). Heminested RT-PCR was performed as described by Heaton *et al.* [5]. The products of positive PCR amplification were purified with a commercial kit (QIAquick® gel extraction KIT, QiagenGmbH, Germany) and were sequenced using the BigDye® Terminator v3.1 cycle sequencing kit (Applied Biosystems, Foster City, CA, USA)

TABLE 1. Case reports of four cases of rabies in humans in Turkey between 2002 and 2006

Case no.	Year	Gender	Province	Case details
TR33	2002	Male	Kilis	A soldier was bitten by an unidentified animal during special duty at night in Kilis. He failed to inform a clinician and did not receive specific anti-rabies prophylaxis. After an undefined incubation period, he developed neurological disease and died. Clinicians suspected rabies and a brain sample was sent to the Rabies Diagnosis Laboratory at Etlik Central Veterinary Control and Research Institute (ECVCRI), Rabies Diagnosis Laboratory. Rabies was confirmed by the fluorescent antibody test and the mouse inoculation test
TR149	2004	Male	Agri	Between 23 August and 3 September, a resident ofthe village of Kazancı (Patnos District) in the East Anatolian Region was bitten on his arm and finger by a fox when hunting. He did not request specific anti-rabies treatment. Clinical signs started 30–35 days later in the final week of September. He was admitted to a number of governmental health units and finally was sent to the Ministry of Health, Ankara Training and Research Hospital. Rabies was considered, because of both clinical signs and history of an animal bite. Samples of saliva and cerebrospinal fluid were sent to ECVCRI on 6 October and corneal impressions were sent on 7 October. Rabies was confirmed on 7 October by hemi-nested RT-PCR and by the fluorescent antibody test on 8 October. The patient died on 8 October, approximately 40 days following exposure
TR40	2006	Female	Adana	A villager was bitten by her dog in the spring but did not seek medical treatment and received no anti-rabies prophylaxis. She developed clinical signs between 35 and 40 days in April and was taken to hospital by members of her family. Rabies was considered, based on clinical presentation and history of a dog bite. Doctors informed ECVCRI and sent saliva, cerebrospinal fluid, corneal impressions and rheum from the eyes. Rabies was confirmed by heminested RT-PCR and the fluorescent antibody test before death
TR3	2006	Male	Aydin	A villager developed clinical signs of rabies and he was transferred to a health unit in Aydin. He did not receive specific anti-rabies prophylaxis. After several days, he died. Doctors suspected rabies, based on clinical presentation. Brain tissue was sent to the local rabies diagnostic centre and rabies was confirmed by the fluorescent antibody test and mouse inoculation test post-mortem. The source of the virus is unknown

according to the manufacturer's instructions. Automated fluorescence sequencing was performed with an ABI PRISM® 310 Genetic Analyzer (Applied Biosystems). The sequences were edited using Bioedit software (http://www.mbio.ncsu.edu/BioEdit/page2.html) and were aligned with the ClustalX1.83 program, version 8 [6]. Further sequences of rabies virus isolates from Turkey (Fig. 1) and neighbouring countries in the south were obtained from GenBank (Table 2). The phylogenetic tree was generated using Phylip, version 3.2, as previously described [7]. One thousand replicates were performed, with values over 700 being considered significant.

A 500 bp partial sequence of the nucleoprotein gene was obtained from each isolate. Sequencing results of the positive primary PCR amplifications confirmed that all four rabies virus isolates from humans were from genotype 1 and were distinct from the fixed Pasteur virus strain. Comparison with a panel of rabies viruses indicated a strong geographical linkage with endemic viruses in the various regions of Turkey.

Molecular epidemiology suggests that the rabies virus isolates identified within Turkey are more related to isolates from the Middle East than to those from Europe [8,9]. A phylogenetic comparison of 37 rabies virus sequences exclusively from Turkey (Fig. 1a) confirms the geographical separation of two groups within the country, namely from eastern and western regions. This comparison confirms previous observations [8].

A larger comparison of a representative panel of Turkish isolates, including the four human virus isolate sequences, with rabies virus sequences from neighbouring countries throughout the Middle East (Table 1, Fig. 1b) suggests that the viruses from western Turkey are distinct from the viruses found in other countries including Israel, Jordan, Lebanon, Saudi Arabia and Iran. The single representative of the eastern Turkish lineage (T149) is found among the Middle East variants, suggesting a common origin of these viruses. As observed in previous studies, there appears to be clustering between viruses from the Eastern Turkish lineage and a rabies virus sequence from Georgia (RV308) to the north-east (Fig. 1b) [8,10]. Furthermore, the small cluster of viruses from southern Turkey, including two human cases, is closely linked to a group of viruses that emerged in the Golan Heights region in 2004. The longer sequence used for this comparison (420 bp) appears to have considerably improved the bootstrap support for this analysis.

Further analysis of these isolates with sequences derived from rabies viruses from the Middle East region revealed that they formed a tight cluster with a group of viruses recently isolated from the Golan Heights region. David *et al.* [9] have already documented the association between this genetic variant and viruses from Turkey. Recent isolations of virus had suggested the emergence of a new variant in northern Israel [11]. This present study suggests that there is a link, possibly associated with the translocation of rabies virus from a source in Turkey, directly south, and emerging as a fresh epizootic in the Golan Heights. These data provide preliminary evidence of long distance translocation of a strain of dog rabies within the Middle East. This rabies virus variant might have originated in north-west Turkey where the western Turkish clade has been endemic at least since 1989 (Fig. 1a) [8,12]. It then appeared in southern Turkey, causing a human fatality in 2002 (TR33). A later case in 2003 in a dog suggests that dogs were the reservoir rather than wildlife.

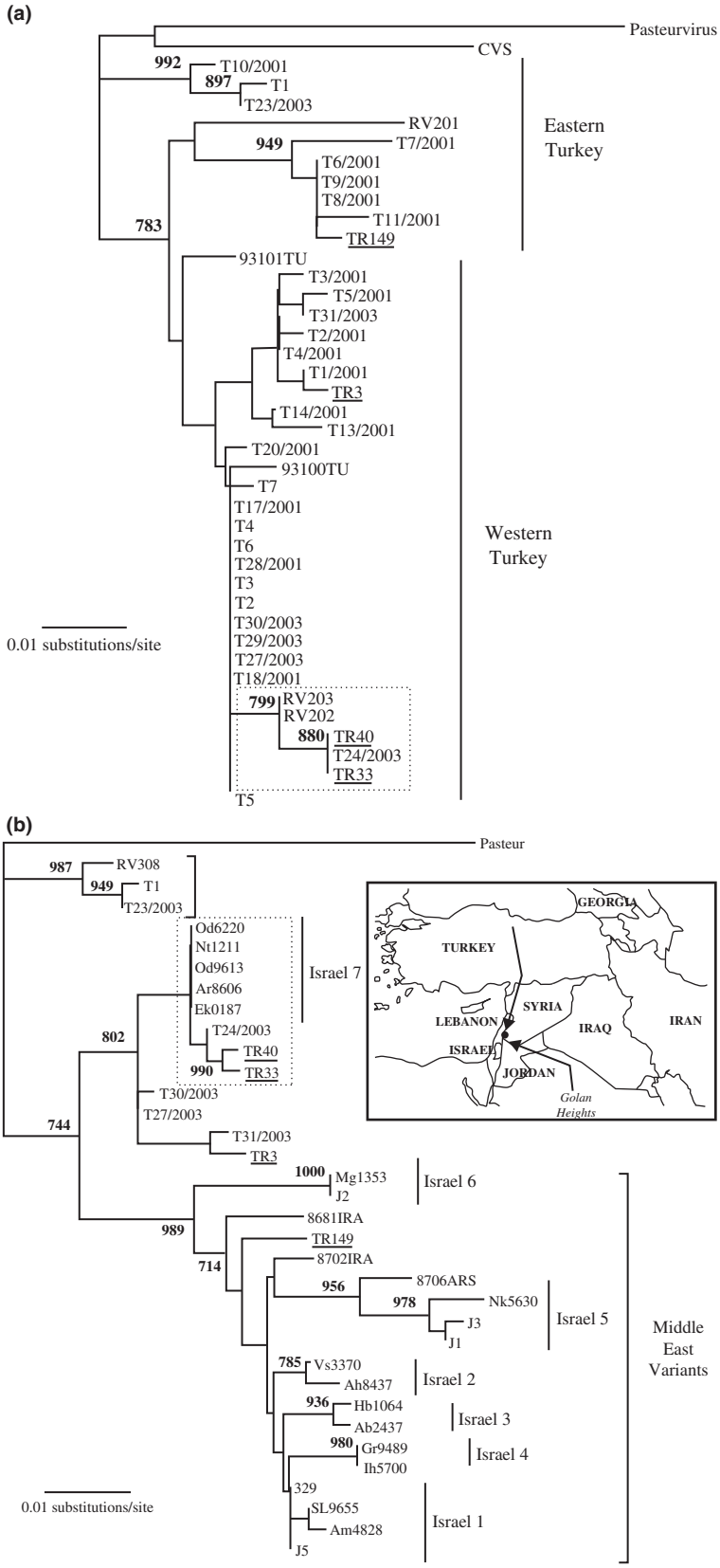

FIG. 1. Phylogenetic analysis of rabies virus (RABV) nucleoprotein sequences derived from four human cases in Turkey. (a) Phylogeny (322 bp) derived from viruses isolated in Turkey. The Pastuer virus (PV) and Challenge Virus Standard (CVS) fixed rabies isolates were used as an outgroup. Bootstrap values >700 were considered significant and are included in the figure. (b) Phylogeny of RABV nucleoprotein sequences (420 bp) derived from viruses isolated from throughout the Middle East. Rabies virus sequences isolated from humans are underlined. The Pastuer virus (PV) was used as an outgroup and bootstrap values greater than 700 are included. Inset provides a map of the Middle East showing the location of countries in close proximity to Turkey. The location of the Golan Heights area is highlighted (arrow) and the possible direction of RABV spread is indicated by a dashed line.

TABLE 2. Isolate details derived from rabies virus from Turkey and neighbouring countries between 1986 and 2006

Sample number	Country	Province/location	Host	Year	Genebank accession number	References
RV201	Turkey	Yavuzeli	Dog	1989	EU180613	This study
RV202	Turkey	Samsun	Dog	1989	AY091608	8
RV203	Turkey	Samsun	Wolf	1989	AY091609	8
T1/2000	Turkey	Manisa	Fox	2000	AY091610	8
T2/2001	Turkey	Izmir	Fox	2001	AY091611	8
T3/2001	Turkey	Izmir	Fox	2001	AY091612	8
T4/2001	Turkey	Manisa	Fox	2001	AY091613	8
T5/2001	Turkey	Manisa	Fox	2001	AY091614	8
T6/2001	Turkey	Erzurum	Fox	2001	AY091615	8
T8/2001	Turkey	Erzurum	Dog	2001	AY091616	8
T8/2001	Turkey	Gaziantep	Bovine	2001	AY091618	8
T9/2001	Turkey	Mardin	Cow	2001	AY091618	8
T10/2001	Turkey	Ardahan	Dog	2001	AY091619	8
T11/2001	Turkey	Ardahan	Dog	2001	AY091620	8
T13/2001	Turkey	Bursa	Dog	2001	AY091621	8
T14/2001	Turkey	Bursa	Dog	2001	AY091622	8
T18/2001	Turkey	Istanbul	Dog	2001	AY091623	8
T18/2001	Turkey	Istanbul	Dog	2001	AY091624	8
T20/2001	Turkey	Istanbul	Dog	2001	AY091625	8
T23/2003	Turkey	Hatay	Cow	2003	AY956352	2
T24/2003	Turkey	Gaziantep	Dog	2003	EU180614	This study
T27/2003	Turkey	Istanbul	Jackal	2003	AY536254	2
T28/2003	Turkey	Istanbul	Jackal	2003	AY536255	2
T29/2003	Turkey	Istanbul	Jackal	2003	AY536256	2
T30/2003	Turkey	Istanbul	Jackal	2003	AY536257	2
T31/2003	Turkey	Aydin	Fox	2003	AY536258	2
93100TU	Turkey	–	Dog	1993	U43014	12
93101TU	Turkey	–	Dog	1992	U43015	12
T1	Turkey	Sanlurfa	Cow	2000	DQ837474	9
T2	Turkey	Istanbul	Dog	2000	DQ837475	9
T3	Turkey	Istanbul	Dog	2000	DQ837476	9
T4	Turkey	Istanbul	Dog	2000	DQ837477	9
T5	Turkey	Istanbul	Dog	2000	DQ837478	9
T6	Turkey	Istanbul	Dog	2000	DQ837479	9
T7	Turkey	Istanbul	Dog	2000	DQ837480	9
TR33	Turkey	Kilis	Human	2002	EU180616	This study
TR149	Turkey	Agri	Human	2004	EU180618	This study
TR3	Turkey	Aydin	Human	2006	EU180615	This study
TR40	Turkey	Adana	Human	2006	EU180617	This study
8681IRA	Iran	–	Dog	1986	U22482	14
8702IRA	Iran	–	Wolf	1987	U22483	14
8706ARS	SaudiArabia	–	Red Fox	1987	U22481	14
RV308	Georgia		Cow	–	AY352497	10
SL9655	Lebanon	–	Stone Marten	1997	DQ837465	11
J1	Jordan	Amman	Goat	1999	DQ837422	11
J2	Jordan	Karak	Donkey	1999	DQ837423	11
J3	Jordan	Irbid	Cow	1998	DQ837424	11
J5	Jordan	Ajloun	Squirrel	1998	DQ8374226	11
Am4828	Israel	Aramsha	Cow	2004	DQ837390	11
329	Israel	Golan Heights	Human	1996	DQ837383	11
Vs3370	Israel	Salama	Fox	1995	DQ837484	11
Ah8437	Israel	Ahihud	Cow	2005	DQ837387	11
Ab2437	Israel	Avihail	Fox	2004	DQ837385	11
Hb1064	Israel	Hebron	Donkey	2002	DQ837414	11
Ih5700	Israel	Iahel	Fox	1997	DQ837419	11
Gr9489	Israel	Grofit	Fox	1998	DQ837411	11
Nk5630	Israel	Neot Hakikar	Fox	2000	DQ837450	11
Mg1353	Israel	Merom Golan	Cow	1997	DQ837441	11
Od6220	Israel	Odem	Wolf	2005	DQ837454	11
Ar8606	Israel	Elrom	Badger	2005	DQ837932	11
Nt1211	Israel	Natur	Cow	2004	DQ837453	11
Od9613	Israel	Odem	Dog	2005	DQ837455	11
Ek0187	Israel	Ein Kinya	Dog	2005	DQ837408	11
Az0298	Israel	Ein Zivan	Dog	2005	DQ837393	11

In 2004, the first case of rabies from this new lineage was reported in the Golan Heights, leading to a sudden increase in animal cases in 2005. Direct translocation of the vector could have achieved the movement from Turkey to the Israel/Syria border. Rabies cases are reported in both Syria and Lebanon, although the number of cases is low [13], most probably as a result of poor surveillance. At present, it is only possible to speculate on the mechanism of movement, although the available phylogenetic evidence suggests that translocation of a new variant of rabies has occurred between Turkey and Israel in recent years.

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Transparency Declaration

The authors have no known conflicting interests within this work.

References

1. Aylan O, Yoruk I, Un H. Rabies in Turkey. *J Etlik Vet Microbiol* 1998; 9: 3–8.
2. Johnson N, Un H, Vos A, Aylan O, Fooks AR. Wildlife rabies in Western Turkey: the spread of rabies through the western provinces of Turkey. *Epidemiol Infect* 2006; 134: 369–375.
3. Dean DJ, Abelseth MK. The fluorescent antibody test. In: Kaplan MM, Koprowski H, eds, *Laboratory techniques in rabies*, 3rd edn. Geneva: WHO, 1973; 73–84.
4. Koprowski H. The mouse inoculation test. In: Meslin FX, Kaplan MM, Koprowski H, eds, *Laboratory techniques in rabies*, 4th edn. Geneva: WHO, 1996; 80–87.
5. Heaton PR, Johnstone P, McElhinney LM, Cowley R, O'Sullivan E, Whitby JE. Heminested PCR assay for detection of six genotypes of rabies and rabies related viruses. *J Clin Microbiol* 1997; 35: 2762–2766.
6. Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG. The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignments aided by quality analysis tools. *Nucleic Acids Res* 1997; 25: 4876–4882.
7. Johnson N, McElhinney LM, Smith J, Lowings P, Fooks AR. Phylogenetic comparison of the genus Lyssavirus using distal coding sequences of the glycoprotein and nucleoprotein genes. *Arch Virol* 2002; 147: 2111–2123.
8. Johnson N, Black C, Smith J *et al.* Rabies emergence among foxes in Turkey. *J Wildl Dis* 2003; 39: 262–270.
9. David D, Hughes GJ, Yakobson BA *et al.* Identification of novel canine rabies virus clades in the Middle East and North Africa. *J Gen Virol* 2007; 88: 967–980.
10. Kuzmin IV, Botvinkin AD, McElhinney LM *et al.* Molecular epidemiology of terrestrial rabies in the former Soviet Union. *J Wildl Dis* 2004; 40: 617–631.
11. David D, Yakobson B, Smith JS, Stram Y. Molecular epidemiology of rabies virus isolates from Israel and other middle-and near-eastern countries. *J Clin Microbiol* 2000; 38: 755–762.
12. Bourhy H, Kissi B, Audry L *et al.* Ecology and evolution of rabies virus in Europe. *J Gen Virol* 1999; 80: 2545–2557.
13. Seimenis A, Morelli D, Mantovani A. Zoonoses in the Mediterranean Region. *Ann Ist Super Sanita* 2006; 42: 437–445.
14. Kissi B, Tordo N, Bourhy H. Genetic polymorphism in the rabies virus nucleoprotein gene. *Virology* 1995; 209: 526–537.

Kinetics of nasopharyngeal shedding of novel H1N1 (swine-like) influenza A virus in an immunocompetent adult under oseltamivir therapy

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Abstract

We describe a patient with confirmed novel H1N1 (swine-like) influenza A virus who had daily nasal swabs tested during oseltamivir therapy. Nasal shedding remained positive for 2 days and became negative on day 3. This report presents the first available data on the kinetics of shedding of this novel virus under antiviral therapy.

Keywords: Kinetics, nasal shedding, oseltamivir, PCR, swine-related influenza

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